

Effect of Phthalate Esters on Reproduction in Rats*

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Introduction

Numerous plastic devices and materials used for medical, dental, and food collection, processing, and packaging are made of poly(vinyl chloride) and plasticized mainly with di-2-ethylhexyl phthalate (DEHP) to impart the desired physical and chemical characteristics. Some finished products may contain more than 40% plasticizer, which may be leached from the material by blood (1) or milk (2) or various other solutions.

DEHP has generally not been considered to be a health hazard because of its low acute oral toxicity (3-10) and because the amount of DEHP leached from plastics is quite small. However, there is evidence that DEHP is not readily hydrolyzed but accumulates in body tissues. When two patients were infused with blood stored in plastic blood bags plasticized with DEHP, Jaeger and Rubin (11) found DEHP in the spleen, liver, lung, and abdominal fat in concentrations ranging from 2.5 to 27 mg-% (dry weight).

The teratogenic effects of several phthalate esters when injected into the yolk sac or allantoic cavity or applied to the chorioallantoic membrane of developing chick embryos have been reported by Haberman and others (12-14). More recently,

Singh et al. (15) have reported the teratogenicity of phthalate esters in rats without regard to the effect these compounds might have on parturition. The present study was undertaken to determine the effects of dibutyl phthalate, dimethyl phthalate, and di-2-ethylhexyl phthalate on embryonic development and parturition.

Materials and Methods

Materials

Three phthalate esters, di-2-ethylhexyl phthalate (DEHP), dimethyl phthalate (DMP), and dibutyl phthalate (DBP) (Monsanto Chemical Co., St. Louis), were included in experiment I. Only DEHP was used in experiment II.

Test animals were adult, virgin female, Sprague-Dawley rats weighing 200 to 220 g. Males of the same strain were used for breeding.

Methods

Six rats, five female and one male, were housed in a large cage in a room kept between 22 and 25°C. Fresh tap water and Lab-Blox (Allied Mills, Inc., Chicago) were provided *ad libitum*. Females were selected for experiment and assigned to test groups after sperm were observed in daily vaginal smears. Smears were obtained by introducing a small drop of clean, fresh tap water into the vagina with a smooth, clean medicine dropper, withdrawing a small liquid sample

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and transferring it to a clean microscope slide. The fresh smears were examined microscopically. If sperm were observed, the female was considered pregnant, this being taken as day one of gestation, and assigned to a test group. The pregnant females were placed in separate cages with *ad libitum* feed and water and left undisturbed except for specified injections until parturition. Five female rats constitute a group.

Phthalates and saline were administered with a sterile syringe and needle by intraperitoneal injection. In experiment I animals were injected at 3, 6 and 9 days of gestation. In experiment II they were injected at 1 or 3, 6 or 9 days of gestation or a combination thereof.

In experiment I, DBP or DEHP was injected at 2 or 4 ml/kg body weight, DMP was injected at 0.5, 1, or 2 ml/kg body weight, and sterile normal saline injected at 4 ml/kg body weight only. In experiment II sterile DEHP and saline were injected at 2 ml/kg body weight. A negative control group was also included. Each animal was then allowed to continue through parturition. When a female died during parturition or only dead young were observed, the uterine horns were exposed to permit the number of resorption sites and retained fetuses to be counted.

For experiment III, the female pups from experiment II were raised to maturity, placed four per cage with a male for 21 days, and then caged separately through parturition to determine whether pups from dams treated with DEHP show normal reproduction.

Results and Discussion

Experiment I

DEHP prevented implantation in 7 out of 10 rats when injected at 3, 6 and 9 days of gestation at the 2 or 4 ml/kg body weight level (Table 1). These levels also have an adverse effect on parturition. Two of the three rats died during parturition (Table 1). Excessive bleeding was noted in all three DEHP-treated females at parturition.

DBP caused a 50% reduction in the number of pups weaned per litter. Two male pups, one from each of two litters in the group treated at the level of 2 ml/kg body weight were born without eyes. In all groups except the group treated with DMP at 2 ml/kg body weight, one or more of the animals did not implant (Table 1).

The differences between the number weaned in the DMP treatments and control are not significant.

Table 1. Experiment I: Effects of phthalate esters on reproduction of rats when injected at 3, 6, and 9 days of gestation.

Compound	Dose level, ml/kg	Number surviving	Number implanting	Average number weaned per litter
DEHP	4	5	1 ^a	—
	2	5	2 ^b	4 ^{+c}
DBP	4	4	3	6.3 ± 1.4
	2	5	4	4.75 ± 1.6
DMP	2	5	5	8.8 ± 1.6
	1	2	1	8.0 ⁺
	0.5	5	4	9.5 ± 3.1
Saline control	4	5	4	10.25 ± 0.5

^a Died at parturition: shed 3 pups, retained 8.

^b One died at parturition: shed 6 pups, retained 7.

^c One litter only.

Experiment II

From the data presented in Table 2, it appears that DEHP has an effect on implantation. Only those groups treated on or before day 6 show animals failing to implant, day 6 normally being considered as the day of implantation.

An effect on parturition can be observed in the groups injected on days 6, 9, 6 and 9, or 3, 6, and 9. Either the mother died or all of the pups died due to poor mothering. Here, as in experiment I, excessive bleeding was noted and suspected to be the cause of death of the one 6 and 9 day mother. The death(s) of the pups in these groups is attributed to the retained fetus(es) which caused the mothers to become ill.

An effect on litter size is not as evident as indicated by the standard error of the mean. These values indicate that there is little chance for significance for either live pups observed or average number weaned, due to the small number of litters. It should be noted, however, that if all animals that im-

Table 2. Experiment II: Effects of DEHP on reproduction of rats when administered at 2 ml/kg body weight and at various stages of gestation.

Day(s) of gestation	Number implanting	Average number live pups observed	Average number of pups weaned	Dams dying or sacrificed at parturition	Retained fetuses
1	5	9.4 ± 0.6	7.2 ± 1.1	0	—
3	4	8.3 ± 1.7	6.7 ± 2.3	0	—
6	4	8.5 ± 3.5	8.5 ± 3.5	2 ^a	5
9	5	11.2 ± 0.86	—	5 ^a	2
3 and 6	3	9.0 ± 1.5	9.0 ± 1.5	0	—
6 and 9	5	11.0 ± 1.9	10.0 ± 1.8	1	8
3, 6, and 9	4	5.0 ^b	5.0	3 ⁺	3
Control	5	11.2 ± 0.4	9.8 ± 1.2	0	—
Negative control	5	10.0 ± 1.1	10.0 ± 0.9	0	—

^a Mothers sacrificed.

^b One litter only.

planted were considered in the average, then the 6, 6 and 9, and 3, 6, and 9 day groups would have averages of 4.2, 8.8, and 1.2, respectively, for the number of live pups observed, far below the control average.

Experiment III

The results in Table 3 show no significant differences at the 5% level between the average litter size of control and treatment groups. This experiment indicates that DEHP has no affect on the reproduction of F₁ females.

Summary and Conclusions

The results of these experiments indicate that DEHP affects implantation and parturition. First, there appears to be an adverse effect on implantation if DEHP is administered during early gestation. This observation is in disagreement with that of Singh et al. (15), who reported that DEHP did not affect implantation. However, their earliest injection would have been equal to our 6-day group, the latest day of gestation this effect was observed. Second, there appears to be an adverse effect on parturition itself when administered at 6 or 9 days of gestation. DEHP caused excessive hemorrhaging

and fetal retention. This observation has not been observed by others because the dams were usually sacrificed 2 to 3 days prepartum.

Although the amount of DEHP administered in this experiment is high, it is possible that much lower levels could be embryotoxic or affect parturition in humans.

Further investigations are needed to determine the minimum levels of DEHP that affect parturition and organogenesis.

Table 3. Reproductive performance of the female offspring from experiment II.

Experiment II treatments (day of gestation injected)	Number of litters	Average number per litter
1	19	11.6 ± 0.8
3	9	12.7 ± 0.9
6	11	11.9 ± 0.9
3 and 6	15	13.2 ± 0.7
6 and 9	10	10.9 ± 0.5
3, 6 and 9	6	14.3 ± 1.4
Control	17	12.8 ± 0.7
Negative control	16	13.1 ± 0.8

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